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THE EGGS OF *AEDES DENTATUS* AND *AEDES FOWLERI* (DIPTERA: CULICIDAE)

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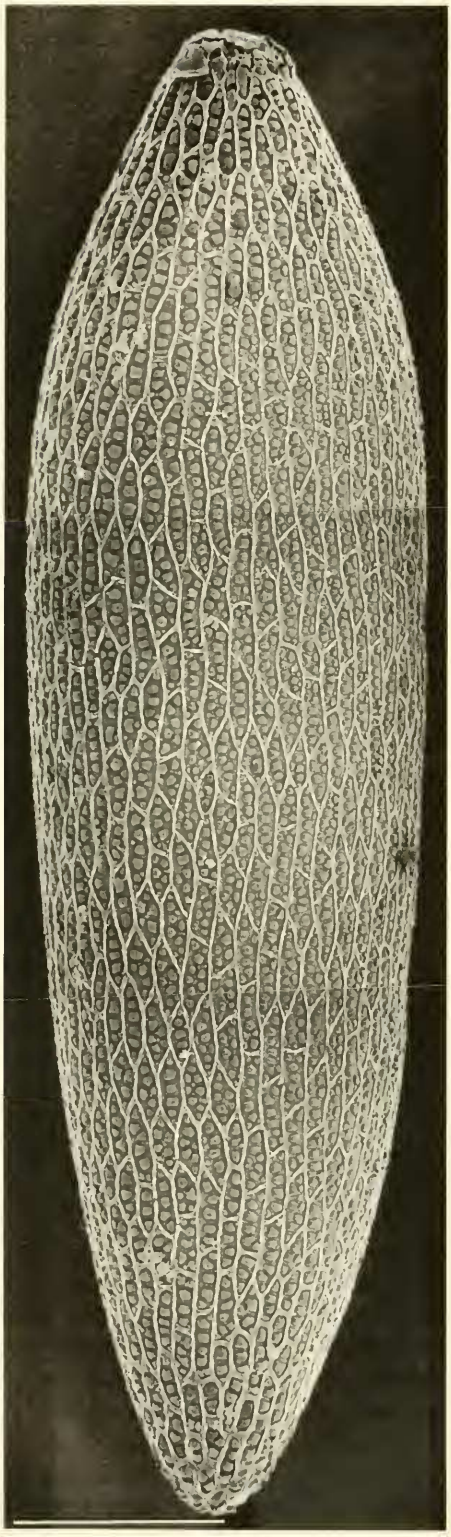
Abstract.—Descriptions illustrated by scanning electron micrographs are given of the eggs of *Aedes* (*Aedimorphus*) *dentatus* and *Ae.* (*Adm.*) *fowleri*. The ventral surface in both species is slightly more curved than the dorsal, and the outer chorionic cells, which are elongated in the longitudinal axis of the egg, are uniform in detailed structure over the entire egg. Each cell in *Ae. dentatus* contains several large tubercles with very few small ones; *Ae. fowleri* cells have several large, central, often partially fused tubercles, surrounded by many peripheral small ones. The micropylar collar is low and very inconspicuous in *Ae. dentatus*, elevated and prominent in *Ae. fowleri*.

Key Words: Insecta, mosquito, egg, scanning electron microscopy

The two *Aedes* (subgenus *Aedimorphus*) species studied in this contribution, *Ae. dentatus* (Theobald) and *Ae. fowleri* (Charmoy) are widely distributed in the Ethiopian Region (Knight and Stone 1977), where both are of concern as vectors of human pathogens. Isolations of Orungo virus at the same time as human isolations have been obtained from *Ae. dentatus* in Nigeria (Tomori and Fabiyi 1977) and, in addition, this species has yielded isolations of Rift Valley fever virus (Meegan and Bailey 1989), as well as Pongola, Semlike Forest, Shokwe and Wesselbron viruses (Karabatsos 1985). *Aedes fowleri* has proven in laboratory experiments to be a competent vector of Rift Valley fever virus (Turell et al. 1988) and also has provided isolations of Pongola, Simbu and Zika viruses (Karabatsos 1985). The larval and adult stages of both species are, of course, known (see Knight and Stone 1977), but no information on the egg of either appears to have been published. As one of us (MJT) was able to collect eggs, we took the opportunity to provide the following descriptions, illustrated by scanning electron micrographs.

Materials and Methods

Aedes dentatus eggs were obtained by decapitation from gravid but unmated females reared after flooding eggs collected by the method of Horsfall (1956) from soil samples taken from natural habitats in Kenya. The infertile eggs so obtained were kept for 24 h on wet filter paper, fixed in alcoholic Bouin's fixative, then sealed in small vials and mailed to Vero Beach. On receipt, eggs were washed in three changes of 80% ethanol to remove picric acid, and were then completely dehydrated in absolute ethanol and dried by the critical point method. To obtain the required orientations on stubs, individual eggs were lifted with a fine artist's brush and touched to sticky tape already fixed and trimmed on the stub surfaces. With



Ae. fowleri, fertile eggs were collected by allowing laboratory colony females to oviposit on damp filter paper. This colony originated from specimens collected in eastern Senegal in 1983 (Turell et al. 1988). Fixation was not required for these specimens, as they resisted desiccation well. Individual live eggs were therefore placed on stubs as already described and, for both species, specimens were then dried finally over calcium chloride (20 min) before being coated with gold and examined in a Hitachi S-510 scanning electron microscope.

All measurements were made from micrographs using a digitizing tablet and SigmaScan software (Jandel Scientific, Corte Madera, CA). Means cited in the text are given ±SE and were derived from an equal number of measurements from 5 eggs of each species. Outer chorionic cell lengths are the dimension in the longitudinal axis of the egg, widths are the circumferential dimension. Tubercles were measured across the widest point, including the base, which is noticeably wider in *Ae. dentatus* than the top. We have used the terminology of Harbach and Knight (1980) and, additionally, the terms "anterior ring" and "outer chorionic cell field" (Linley 1989) and "micropylar dome" (Linley et al. 1991).

Results

Aedes (*Aedimorphus*) *dentatus*
(Figs. 1–3)

Size: as in Table 1.

Color: dull black.

Shape, overall appearance: cigar-shaped, widest at about anterior 0.25, anterior end distinctly conical, posterior taper slight from widest point to posterior 0.3, then more rapid, posterior end slightly pointed (Fig. 1). Lateral view shows ventral surface scarcely more curved than dorsal (Fig. 2a). Outer chorionic cells regular, easily visible, elon-

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Fig. 1. *Aedes dentatus.* Entire egg, ventral view, anterior end at top. Scale = 100 μm.

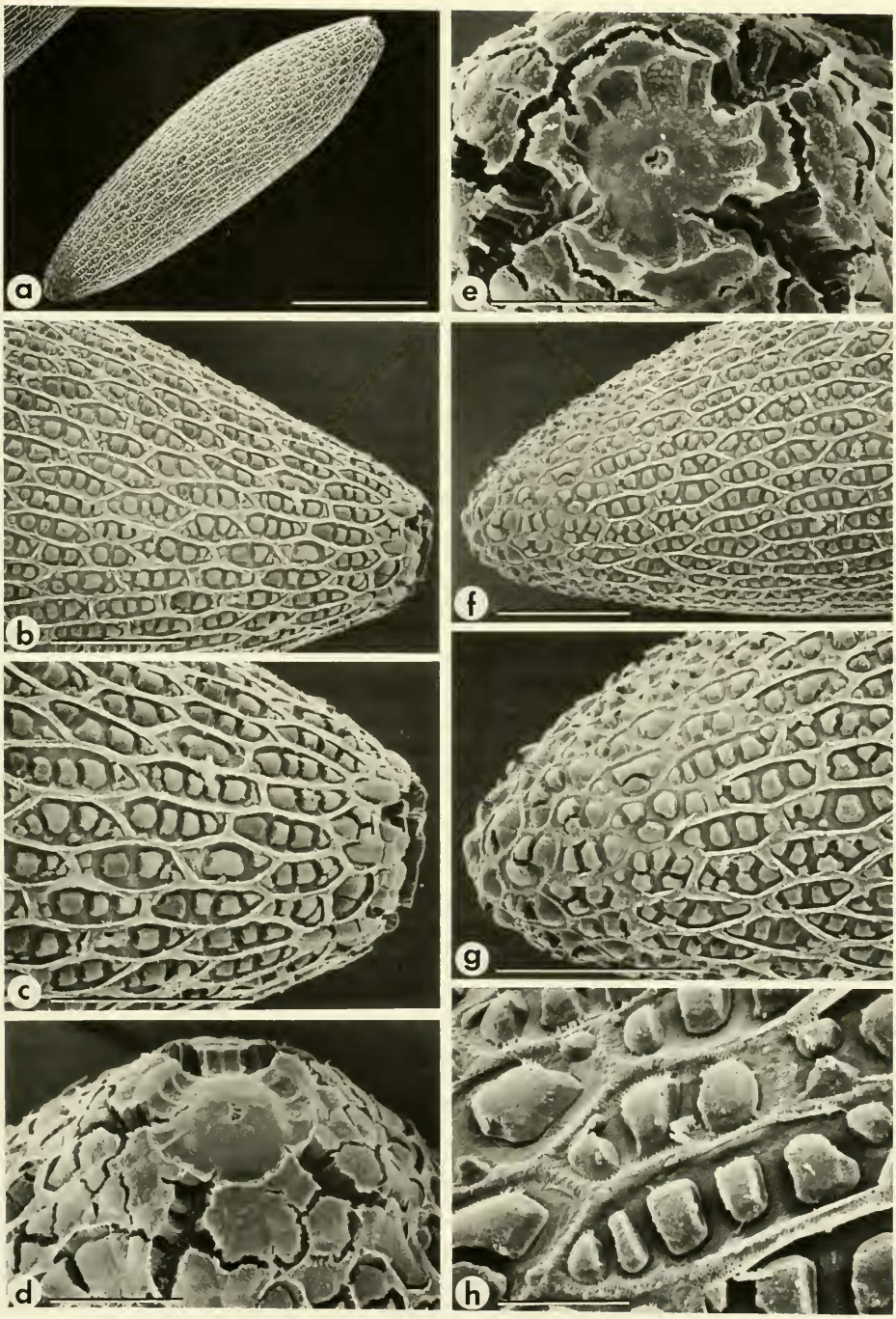

Fig. 2. *Aedes dentatus*. (a) Entire egg, lateral view, ventral side at top, anterior end at right; (b) anterior end, lateral view, ventral side at top; (c) anterior end, chorionic cell detail; (d) micropylar apparatus, showing disk and indistinct dome; (e) micropylar apparatus, detail of collar inner wall, disk surface; (f) posterior end, lateral view, ventral side at top; (g) posterior end, chorionic cell detail. Scale = 200 μm (a), = 50 μm (b, c, f, g), = 20 μm (d, e), = 10 μm (h).

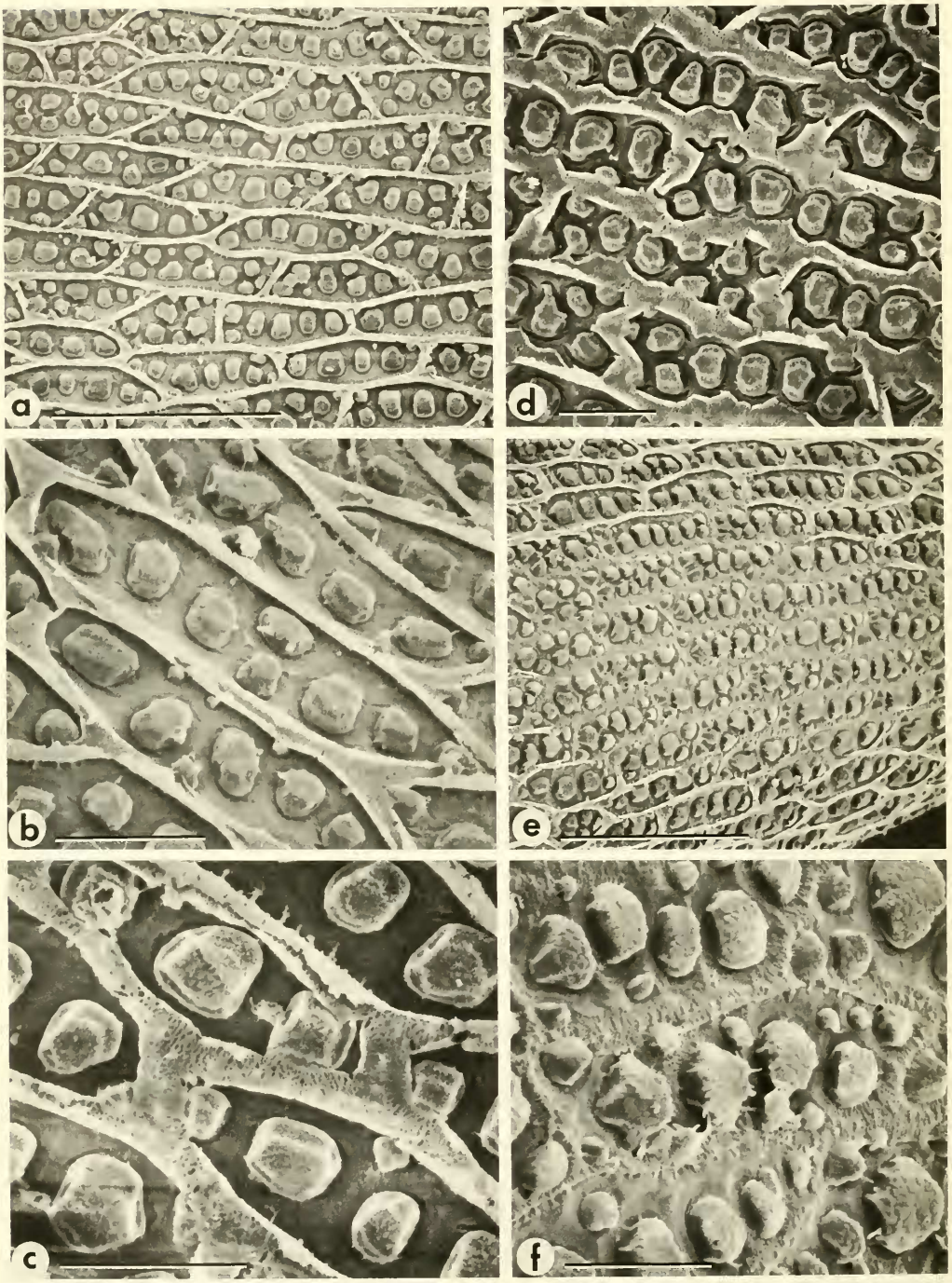

Fig. 3. *Aedes dentatus*. (a) Outer chorionic cells, ventral surface, middle of egg; (b) detail, chorionic cells and tubercles; (c) detail, tubercles and outer chorionic reticulum; (d) atypical chorionic reticulum seen in some eggs on dorsal surface; (e) lateral patch, as seen in a few eggs, with atypical reticulum; (f) detail, atypical reticulum. Scale = 50 μm (a, e), = 10 μm (b, c, d, f).

Table 1. Dimensions of eggs of *Ae. dentatus* (n = 12) and *Ae. fowleri* (n = 10).

Species	Length (μm)		Width (μm)		L/W ratio	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Ae. dentatus	819.2 ± 8.4	766.4–876.2	237.2 ± 2.8	223.4–251.6	3.46 ± 0.06	3.15–3.78
Ae. fowleri	751.8 ± 10.3	681.8–795.4	234.5 ± 3.0	218.2–249.9	3.21 ± 0.05	2.99–3.31

gate in longitudinal axis of egg and each with several tubercles, reticulum narrow (Fig. 1). Collar of micropyle very indistinct.

Chorion, ventral, lateral and dorsal surfaces: all surfaces very similar (Figs. 1, 2a). Outer chorionic cell length, 23.8–45.2 μm (mean 36.1 ± 1.5 μm, n = 15), greater than width, 8.9–12.4 μm (mean 10.6 ± 0.2), length/width ratio 2.17–4.55 (mean 3.43 ± 0.18). Cell shape pentagonal or quadrilateral, longitudinal corners often very narrowly pointed (Figs. 1, 3a), cell fields 1.2–2.2 μm less in each dimension. Tubercles in each cell medium sized, a few small (Fig. 3a), located generally in the central part of the cell field, but a few peripherally positioned, sometimes fused with reticulum (Fig. 3a, b). Number of tubercles 4–13 (mean 8.4 ± 0.4, n = 25), diameter 1.4–5.7 μm (mean 3.2 ± 0.1 μm, n = 50), shapes irregular, tending to be rectangular, the base of each tubercle around some of its sides conspicuously wider than the top (Fig. 3a, b, c). Bases fairly smooth, tops of some with scarcely discernible nodular sculpturing (Fig. 3b, c). Chorionic reticulum 1.2–2.4 μm wide, moderately raised, consisting of a very fine meshwork, frequently folded over at the edges, with central line of papillae (Fig. 3c). Edges of reticulum in some places connected to cell floor by very thin pillars (Fig. 3b, c). On dorsal surface, a few eggs with areas of chorion in which reticulum variable in width but considerably wider (2.4–4.1 μm) overall, appearing ragged (Fig. 3d). Patches of chorion occasionally seen on some eggs in which reticulum again atypical (Fig. 3e), meshwork closely applied to cell surface, edges not raised, central papillae and small pillars along edges more prominent (Fig. 3f).

Anterior end, micropyle: chorionic cells smaller near anterior end, narrower, tubercles fewer (Fig. 2b), but structure relatively little modified except immediately posterior to micropylar collar, where cells very small, fields almost completely filled by tubercles (Fig. 2c). Collar of micropyle discontinuous, gaps present in all eggs examined (Fig. 2d, e), posterior edge very difficult to distinguish, invariably cracked and fused with adjacent cells (Fig. 2c, d). Height of collar (where recognizable) 1–4 μm, width 27–34 μm, wall width (where present) 1–6 μm, surface slightly rough (Fig. 2e). Collar internal diameter 24–26 μm, interior wall excavated, surface rough, nodular (Fig. 2e). Micropylar disk fairly prominent, diameter 13–15 μm, edges clearly raised in some specimens (Fig. 2d), dome inconspicuous (Fig. 2d, e), diameter about 10 μm, orifice 2.4 μm wide.

Posterior end: chorionic cells smaller towards posterior end, tubercles fewer (Fig. 2g), reticulum often becoming appressed to cell floor, central papillae more prominent (Fig. 2g, h). Tubercles often partially fused in cells very near end of egg, extreme end cells with a single, smooth tubercle that completely fills field (Fig. 2g).

Aedes (*Aedimorphus*) *fowleri*
(Figs. 4–6)

Size: as in Table 1.

Color: matte black.

Shape, overall appearance: broadly cigar-shaped in ventral and dorsal view (Fig. 4), ventral side somewhat more curved in lateral view (not shown). Widest at about anterior 0.3, anterior end only slightly conical, little posterior taper until posterior 0.25, then taper greatly increased (Fig. 4). Outer chorionic cells longer than broad, reticulum

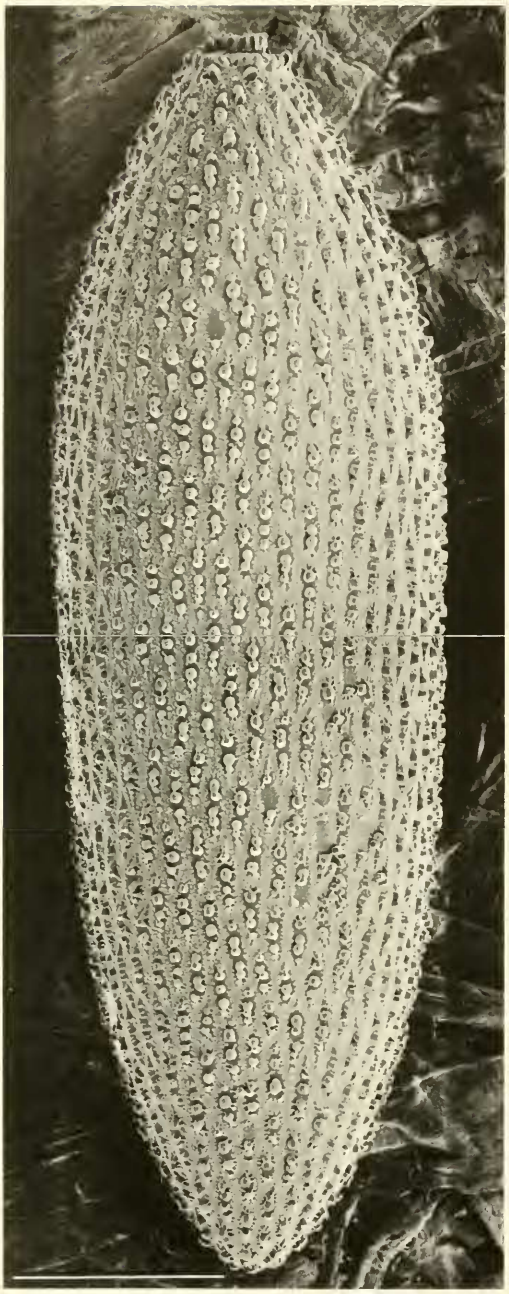

Fig. 4. *Aedes fowleri.* Entire egg, ventral view, anterior end at top. Scale = 100 μm.

fairly wide and boundaries of cells not distinct, several large tubercles visible in each cell, aligned longitudinally (Fig. 4). Micropylar collar conspicuous, sides erect, not conforming to taper of egg (Fig. 4).

Chorion, ventral, lateral and dorsal surfaces: all surfaces very similar (Fig. 5a, c). Outer chorionic cells elongate, length 28.6–51.2 μm (mean 43.4 ± 1.7 μm, n = 15) greater than width, 11.8–15.9 μm (mean 13.8 ± 0.3 μm), length/width ratio 1.98–3.75 (mean 3.15 ± 0.13). Shape of cells hexagonal or pentagonal, anterior and posterior corners often very pointed (Fig. 5a, b), cell fields 2–4 μm less in each dimension. Large tubercles 4–7 in number (mean 5.2 ± 0.3, n = 15), more or less round, arranged in line in central longitudinal axis of cell (Fig. 5a, b), diameter 1.6–5.4 μm (mean 3.6 ± 0.1 μm, n = 50). Many tubercles separate, but multiples formed of 2 or 3 contiguous or partially fused tubercles quite common (Fig. 5a, b, c), largest tubercles usually in middle of cell. In detailed structure each tubercle with base sometimes slightly larger than top, sides of tubercle vertical or almost so, walls rough (Fig. 5b, e, f), top domed, sculptured with small, flat nodules (Fig. 5d, e, f). Small tubercles 15–32 in number (mean 22.9 ± 1.1, n = 15), diameter 0.3–1.9 μm (mean 1.0 ± 0.04 μm, n = 50), fairly evenly spaced around periphery of cell, sometimes not touching reticulum, but often touching it and overlain by its meshwork (Fig. b, d, f). Small tubercles more or less round, low, smooth-surfaced (Fig. 5b, d, e), many with bridges extended to nearby large tubercles (Fig. 5b, c, d, e). Outer chorionic reticulum low, flat, fairly wide, 2.0–4.3 μm, consisting in most places of an intricate and delicate mesh appressed to the cell surface (Fig. 5c, d, f), but often in some places raised to produce perforations (Fig. 5d, e), both forms often found round same cell (Fig. 5b, e). Meshwork with a sometimes indistinct central line of tiny papillae (Fig. 5c, d).

Anterior end, micropyle: chorionic cells progressively smaller in size approaching anterior end, large and small tubercles fewer (Fig. 6a), the former tending to be more abutting or fused. Anterior ring well developed, diameter 51–54 μm, width 9.5–14.0 μm, tubercles in outer ring anteriorly curved,

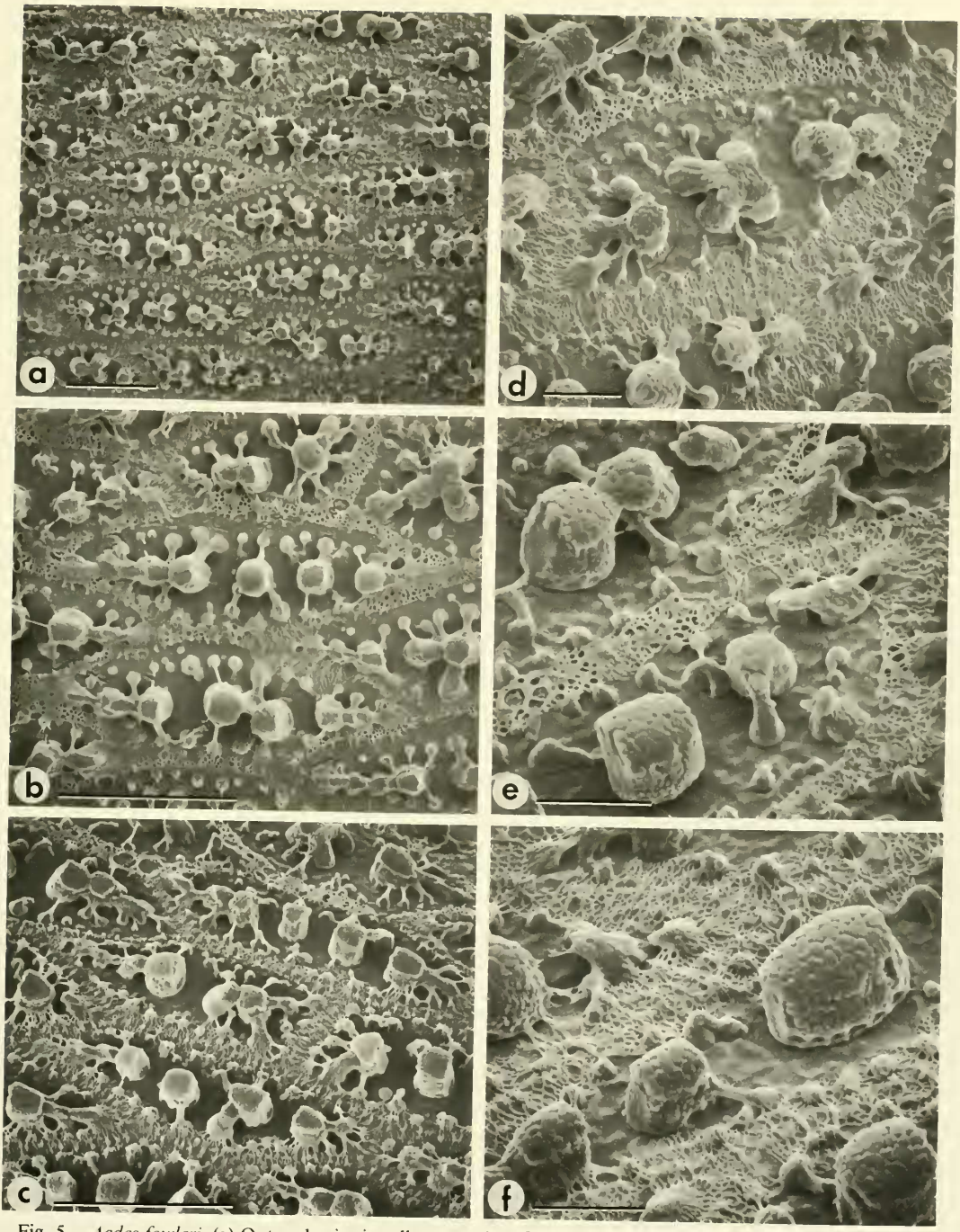

Fig. 5. *Aedes fowleri.* (a) Outer chorionic cells, ventral surface, middle of egg; (b) detail, chorionic cells and tubercles; (c) outer chorionic cells, dorso-lateral surface, middle of egg; (d) detail, single chorionic cell, showing fused tubercles, reticulum mostly of flat, unperforated type; (e) detail, large and small tubercles, some reticulum of perforated type; (f) extreme detail, large tubercles, meshwork of reticulum. Scale = 20 μm (a, b, c), = 5 μm (d, e, f).

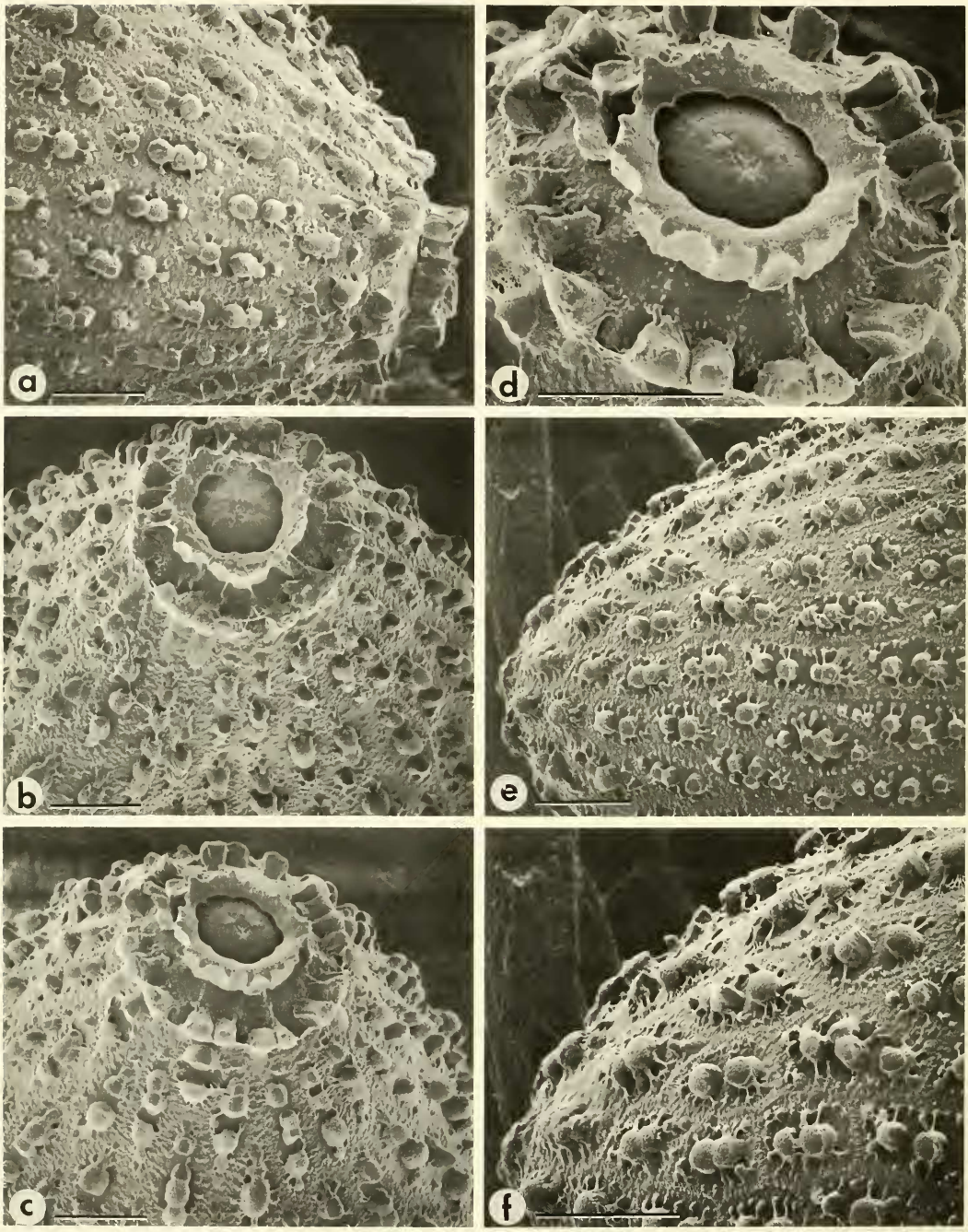

Fig. 6. *Aedes fowleri*. (a) Anterior end, lateral view, ventral surface at top; (b) anterior ring and micropylar apparatus, collar with single gap; (c) anterior ring and micropylar apparatus, collar continuous; (d) detail, micropylar apparatus; (e) posterior end, lateral view, ventral surface at top; (f) posterior end, chorionic cell detail. Scale = 20 μm.

tops blunt, rather square (Fig. 6b, c, d). Micropylar collar prominent, erect or more often outwardly flared (Fig. 6a, c, d), usually continuous (Fig. 6c) but a small gap occasionally present (Fig. 6b), height 5.5–10.0 μm, outer wall fluted, fairly smooth (Fig. 5d). Collar diameter 23–31 μm, anterior wall downwardly sloped towards interior (Fig. 6d), width 2.3–6.5 μm, collar internal diameter 19–22 μm. Micropylar disk 17–19 μm in diameter, edges very indistinct, surface slightly rough, dome only slightly raised, edge very obscure, diameter about 11 μm, micropylar orifice trilobed, diameter 2.5 μm.

Posterior end: chorionic cells smaller, narrower, large and small tubercles fewer (Fig. 6e), large ones contiguous or fused, cell fields obliterated or almost so in most posterior cells (Fig. 6f). Structure of reticulum often indistinct in cells at extreme end of egg, surface appearing smoother (Fig. 6f).

Discussion

Apart from the 2 species of *Aedimorphus* considered here, the eggs of only 2 others in this subgenus appear to have been described. *Aedes vexans* (Meigen) was examined in several earlier studies in which the outer chorion was stripped away before the chorionic cell outlines were recorded either by phase contrast microscopy (Craig and Horsfall 1960, Myers 1967, Kalpage and Brust 1968), or scanning electron microscopy (Horsfall et al. 1970). However, the intact outer chorion, as well as other details of the undamaged egg, have only recently been illustrated (Linley 1990). Reinert (1972) resourcefully extracted 3 eggs of *Aedes domesticus* (Theobald) from the abdomen of a museum specimen and provided a brief description illustrated by good line drawings of the reticular chorionic pattern.

To the extent that any common characteristics can be observed in these eggs, they appear to be as follows. Ventral surfaces are slightly more curved, dorsal surfaces flatter (*Ae. domesticus* may be the exception), this being most pronounced in *Ae. vexans* (Linley 1990), less so in *Ae. fowleri,* and very little in *Ae. dentatus.* Anterior ends tend to be noticeably conical. The chorionic cells are very uniform in structure over the entire egg, and are relatively simple in shape, elongate in the longitudinal axis of the egg, with often sharply narrowed, pointed anterior and posterior corners. *Aedes domesticus* is interesting in that while the cells in about the anterior and posterior 0.25 are greatly elongate longitudinally, those in the middle 0.25 are longer circumferentially, with transitional types grading to the anterior and posterior areas (Reinert 1972). In the structure of the tubercles, *Ae. dentatus* resembles *Ae. vexans*; only the occasional tubercle in a cell is small and the large tubercles are rather irregular in shape, many tending to be rectangular and their bases larger than their tops. *Aedes fowleri* is different in that its cells have many small, peripheral tubercles and the large tubercles are more or less round without expanded bases. No information is available for *Ae. domesticus.* A point of marked difference between species is the anterior end and micropylar apparatus. *Aedes fowleri* possesses a well developed anterior ring, but in *Ae. vexans* it is poorly formed and often incomplete, while in *Ae. dentatus* it is absent (no details of the anterior end have been provided for *Ae. domesticus*). In similar order of development, the micropylar collar in *Ae. fowleri* is very prominent, less so in *Ae. vexans* and very inconspicuous in *Ae. dentatus.*

Acknowledgments

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Literature Cited

Craig, G. B., Jr. and W. R. Horsfall. 1960. Eggs of floodwater mosquitoes. VII. Species of *Aedes* common in the southeastern United States (Diptera: Culicidae). Annals of the Entomological Society of America 53: 11–18.

Kalpage, K. S. and R. A. Brust. 1968. Mosquitoes of Manitoba. I. Descriptions and a key to *Aedes* eggs (Diptera: Culicidae). Canadian Journal of Zoology 46: 699–718.

Karabatsos, N. 1985. (Ed.) International Catalog of Arboviruses Including Certain Other Viruses of Vertebrates, ed. 3. American Society of Tropical Medicine and Hygiene, San Antonio, Texas. 1,147 pp.

Knight, K. L. and A. Stone. 1977. A Catalog of the Mosquitoes of the World (Diptera: Culicidae). Entomological Society of America, The Thomas Say Foundation. Vol. VI, 611 pp.

Harbach, R. E. and K. L. Knight. 1980. Taxonomists' Glossary of Mosquito Anatomy. Plexus Publishing Inc., Marlton, New Jersey. 415 pp.

Horsfall, W. R. 1956. A method for making a survey of floodwater mosquitoes. Mosquito News 16: 66–71.

Horsfall, W. R., F. R. Voorhees, and E. W. Cupp. 1970. Eggs of floodwater mosquitoes. XIII. Chorionic sculpturing. Annals of the Entomological Society of America 63: 1709–1716.

Linley, J. R. 1989. Comparative fine structure of the eggs of *Aedes albopictus, Aedes aegypti* and *Aedes bahamensis* (Diptera: Culicidae). Journal of Medical Entomology 26: 510–521.

Linley, J. R. 1990. Scanning electron microscopy of the eggs of *Aedes vexans* and *Aedes infirmatus* (Diptera: Culicidae). Proceedings of the Entomological Society of Washington 92: 685–693.

Linley, J. R., M. J. Geary, and R. C. Russell. 1991. The eggs of *Aedes funereus, Aedes notoscriptus* and *Aedes alternans* (Diptera: Culicidae). Proceedings of the Entomological Society of Washington 93: 592–612.

Meegan, J. M. and C. L. Bailey. 1989. Rift Valley Fever, pp. 51–76. *In* Monath, T. P., ed., The Arboviruses: Epidemiology and Ecology. Vol IV. CRC Press, Boca Raton, Florida.

Myers, C. M. 1967. Identification and descriptions of *Aedes* eggs from California and Nevada (Diptera: Culicidae). Canadian Entomologist 99: 795–807.

Reinert, J. F. 1972. Description of the egg of *Aedes* (*Aedimorphus*) *domesticus* (Theobald) (Diptera: Culicidae). Mosquito Systematics 4: 60–62.

Tomori, O. and A. Fabiyi. 1977. Orungo virus, a new agent from mosquitoes and man in Uganda and Nigeria. Nigerian Medical Journal 7: 5–8.

Turell, M. J., M. E. Faran, M. Cornet, and C. L. Bailey. 1988. Vector competence of Senegalese *Aedes fowleri* (Diptera: Culicidae) for Rift Valley fever virus. Journal of Medical Entomology 25: 262–266.